UNCLASSIFIED

AD 400 315

Reproduced
by the

ARMED SERVICES TECHNICAL INFORMATION AGENCY
ARLINGTON HALL STATION
ARLINGTON 12, VIRGINIA

UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

Final *REPORT ON*

CONTRACT NO DA -92-557-FEC-34626

INCLUSIVE DATES 15 February 1962 *TO* 14 February 1963

400315

SUBJECT OF INVESTIGATION

ELECTRON MICROSCOPE STUDY ON THE

INFECTIOUS HEPATITIS

CATALOGED BY ASTIA
AS AD No. 400 315

RESPONSIBLE INVESTIGATOR

Dr. Gonpachiro Yasuzumi

Professor of Anatomy
Nara Medical College
Kashihara, Nara, Japan

APR 9 1963
TISIA

U.S. Army Research & Development Group (9852) (Far East)
Office of the Chief of Research and Development
United States Army
APO 343

AD Div 16/2

Nara Medical College, Nara, Japan
ELECTRON MICROSCOPE STUDY ON THE INFECTIOUS HEPATITIS by Gonpachiro Yasuzumi.
Final Report No. 2, 15 Feb 62 - 14 Feb 63. 8 p. illus. tables, 16 refs.
(Contract DA 92-557-FEC-34626) Unclassified report

Biopsy materials from human liver of kidney in cases of infectious hepatitis, serum hepatitis, hemolytic jaundice, chronic non-specific hepatitis, and familial non-hemolytic jaundice were studied in thin sections with the electron microscope. The cases of infectious hepatitis and serum hepatitis showed cytoplasmic particles suggesting a virus etiology, but it was impossible to identify characteristic particles in other cases. The ultrastructure of cellular alterations accompanying infection by the virus and other hepatotoxic agents was demonstrated and discussed in detail. Further study will afford

UNCLASSIFIED

1. Electron microscopes
2. Hepatitis virus

I. Title: Electron microscopes - Hepatitis virus
II. Yasuzumi, Gonpachiro
III. U.S. Army R&D Gp (FE), OCRD, DA, Wash, D. C.
IV. Contract DA 92-557-FEC-34626

Armed Services Technical Information Agency

UNCLASSIFIED

AD Div 16/2

Nara Medical College, Nara, Japan
ELECTRON MICROSCOPE STUDY ON THE INFECTIOUS HEPATITIS by Gonpachiro Yasuzumi.
Final Report No. 2, 15 Feb 62 - 14 Feb 63. 8 p. illus. tables, 16 refs.
(Contract DA 92-557-FEC-34626) Unclassified report

Biopsy materials from human liver of kidney in cases of infectious hepatitis, serum hepatitis, hemolytic jaundice, chronic non-specific hepatitis, and familial non-hemolytic jaundice were studied in thin sections with the electron microscope. The cases of infectious hepatitis and serum hepatitis showed cytoplasmic particles suggesting a virus etiology, but it was impossible to identify characteristic particles in other cases. The ultrastructure of cellular alterations accompanying infection by the virus and other hepatotoxic agents was demonstrated and discussed in detail. Further study will afford

UNCLASSIFIED

1. Electron microscopes
2. Hepatitis virus

I. Title: Electron microscopes - Hepatitis virus
II. Yasuzumi, Gonpachiro
III. U.S. Army R&D Gp (FE), OCRD, DA, Wash, D. C.
IV. Contract DA 92-557-FEC-34626

Armed Services Technical Information Agency

UNCLASSIFIED

AD Div 16/2

Nara Medical College, Nara, Japan
ELECTRON MICROSCOPE STUDY ON THE INFECTIOUS HEPATITIS by Gonpachiro Yasuzumi.
Final Report No. 2, 15 Feb 62 - 14 Feb 63. 8 p. illus. tables, 16 refs.
(Contract DA 92-557-FEC-34626) Unclassified report

Biopsy materials from human liver of kidney in cases of infectious hepatitis, serum hepatitis, hemolytic jaundice, chronic non-specific hepatitis, and familial non-hemolytic jaundice were studied in thin sections with the electron microscope. The cases of infectious hepatitis and serum hepatitis showed cytoplasmic particles suggesting a virus etiology, but it was impossible to identify characteristic particles in other cases. The ultrastructure of cellular alterations accompanying infection by the virus and other hepatotoxic agents was demonstrated and discussed in detail. Further study will afford

UNCLASSIFIED

1. Electron microscopes
2. Hepatitis virus

I. Title: Electron microscopes - Hepatitis virus
II. Yasuzumi, Gonpachiro
III. U.S. Army R&D Gp (FE), OCRD, DA, Wash, D. C.
IV. Contract DA 92-557-FEC-34626

Armed Services Technical Information Agency

UNCLASSIFIED

AD Div 16/2

Nara Medical College, Nara, Japan
ELECTRON MICROSCOPE STUDY ON THE INFECTIOUS HEPATITIS by Gonpachiro Yasuzumi.
Final Report No. 2, 15 Feb 62 - 14 Feb 63. 8 p. illus. tables, 16 refs.
(Contract DA 92-557-FEC-34626) Unclassified report

Biopsy materials from human liver of kidney in cases of infectious hepatitis, serum hepatitis, hemolytic jaundice, chronic non-specific hepatitis, and familial non-hemolytic jaundice were studied in thin sections with the electron microscope. The cases of infectious hepatitis and serum hepatitis showed cytoplasmic particles suggesting a virus etiology, but it was impossible to identify characteristic particles in other cases. The ultrastructure of cellular alterations accompanying infection by the virus and other hepatotoxic agents was demonstrated and discussed in detail. Further study will afford

UNCLASSIFIED

1. Electron microscopes
2. Hepatitis virus

I. Title: Electron microscopes - Hepatitis virus
II. Yasuzumi, Gonpachiro
III. U.S. Army R&D Gp (FE), OCRD, DA, Wash, D. C.
IV. Contract DA 92-557-FEC-34626

Armed Services Technical Information Agency

UNCLASSIFIED

a further approach to the clarification of the genesis and development of the particles mentioned above. (Author)

UNCLASSIFIED

DESCRIPTORS

Hepatitis
Electron microscopes

UNCLASSIFIED

a further approach to the clarification of the genesis and development of the particles mentioned above. (Author)

UNCLASSIFIED

DESCRIPTORS

Hepatitis
Electron microscopes

UNCLASSIFIED

a further approach to the clarification of the genesis and development of the particles mentioned above. (Author)

UNCLASSIFIED

DESCRIPTORS

Hepatitis
Electron microscopes

UNCLASSIFIED

a further approach to the clarification of the genesis and development of the particles mentioned above. (Author)

UNCLASSIFIED

DESCRIPTORS

Hepatitis
Electron microscopes

UNCLASSIFIED

D-I-S-T-R-I-B-U-T-I-O-N

The distribution of this report as made by USA R&D Gp (FE) is as follows:

Army Research Office, OCRD, Washington 25, D. C.	(2)
Army Attache, American Embassy, Tokyo, Japan	(1)
U.S. Army Medical R & D Command	(4)
ASTIA	(10)
Office of Primary Scientific Liaison	(1)
Offices of Scientific Cognizance	(/)

The Abstract of Final Report No. 2.

Biopsy materials from human liver or kidney in cases of infectious hepatitis, serum hepatitis, hemolytic jaundice, chronic non-specific hepatitis, and familial non-hemolytic jaundice were studied in thin sections with the electron microscope. The cases of infectious hepatitis and serum hepatitis showed cytoplasmic particles suggesting a virus etiology, but it was impossible to identify characteristic particles in other cases. The ultrastructure of cellular alterations accompanying infection by the virus and other hepatotoxic agents was demonstrated and discussed in detail. Further study will afford a further approach to the clarification of the genesis and development of the particles mentioned above.

CONTENT

1. Purpose of the Investigation

The purpose of the investigation is to make an electron microscope study on biopsy materials of human liver or kidney in cases of infectious hepatitis and other diseases. An attampt has been made to clarify what has been left unsettled, e.g., how multiplication of the virus particles is carried out, in what relationship cell organelles stand with the infection, and comparison of the fine structure in the cases of infectious hepatitis and other liver diseases.

2. Materials and Methods

The laboratory tests were previously made as follows: Van den Bergh, direct and indirect; icteric index; Kunkel; thymol unit; CCF; BSP; total protein; cholesterol; ester type cholesterol; alkaline phosphatase; syphilis test; erythrocytes; leucocytes; haemoglobin index; etc. A diagnosis was made sure on light microscope examinations of biopsy materials or ventrotomy.

The biopsy materials of the liver or kidney from patients were available for electron microscopy. The specimens were cut with a razor into slices about 3 mm. in thickness, and immersed for 15 minutes in 1% osmium tetroxide buffered to pH 7.4 with the Michaelis veronal-acetate buffer. These slices were then cut into smaller blocks ca. 1 mm. in thickness and fixation was continued to a total of 60 to 90 minutes. After fixation, the specimens were directly dehydrated in a series of increasing concentrations of ethyl alcohol, and embedded in a mixture of methyl and n-butyl methacrylates, or in Epon epoxy resin. Sections were obtained by using the Porter-Blum microtome equipped with a glass knife, and mounted on copper grids coated with formvar. The sections were then stained according to a slight modification of Watson's lead acetate procedure or uranyl acetate, and a thin coat of carbon evaporated onto them. They were examined in an Akashi TRS-50E$_1$, TRS-60B or TRS-80 electron microscope or an electron microscope of the Japan Electron Company, model JEM-6C.

3. Results

The electron microscopic patterns of biopsy specimens of the liver obtained from two cases of non-infectious hepatitis will be described in the present report. The materials were fixed with the methods mentioned above, embedded in Epon epoxy resin, and examined with the electron microscope, without staining.

One patient, a 23-year-old man, was admitted to Nara

Medical University Hospital because of jaundice which had developed from three years ago. The laboratory tests revealed the following: erythrocytes 3550,000; leucocytes 4,000; haemoglobin index 0.88; icteric index 35; bilirubin direct 0.9 mg/dl, indirect 3.6 mg/dl; Van den Bergh direct (-), indirect (+); CCF (+++); BSP 2%; syphilis test (-). A diagnosis of hemolytic jaundice was made on light examinations and results of the tests.

The other patient, a 25-year-old man, was admitted to Nara Medical University Hospital because of jaundice which had developed three months ago. The laboratory tests revealed the following: erythrocytes 5260,000; leucocytes 6,400; haemoglobin index 0.98; icteric index 20; CCF (+++); BSP 52%; syphilis test (-). A diagnosis of chronic hepatitis was made on light examinations and results of the tests.

The first case (Figs. 1-3)

The glycogen granules appear as light spots duing to nonstaining with heavy metals. Numerous mitochondria in small size are found in the cytoplasm of the parenchymal cell. They are filled with homogeneous matrix, being devoid of almost their cristae. Palade ribonucleoprotein particles (ribosomes) appear isolated or attached to the outer surfaces of the endoplasmic reticulum. The ribosomes are characteristically found to have been arranged along the outer surfaces of the mitochondria (Fig. 1). Numerous dense particle aggregates have been found in the cytoplasm, although only an aggregate is demonstrated in Fig. 1. It is clearly understandable that the particles represent ferritin particles in the parenchymal cells of human liver (1). The cytoplasm of these liver cells also contain often roughly oval- or round-shaped bodies which are filled with a homogenously dense particles and are surrounded by an apparently single membrane of greater density. These bodies correspond in size and structure to the so called lysosomes which possess high levels of acid phosphatase activity. A roughly round body less dense and more homogeneous than the lysosome appears sometimes in the cytoplasm, being surrounded by an apparently single membrane and the matrix being divided by irregularly running dense lines into many parts. This body is assumed to be a lysosomal derivative (2). Virus-like dense particles have never been observed in the nuclei as well as in the cytoplasm.

The second case (Figs. 4-6)

The glycogen granules appear also as light spots in the parenchymal cells. Numerous ribosomes can be seen in the parenchymal cells and the Kupffer's cell, showing often

definite arrangement in a rosette form. Mitochondria are larger in size as compared with those in the first case. A round body containing homogeneous matrix may be a lysosome. Two dense bodies composed of dense particles about 60 A in diameter are found in a vesicle. These particles correspond to ferritin particles in size and density. Virus-like, dense particles have never been observed in this case.

4. Discussion

Several authors (3-6) have indicated that infectious hepatitis is caused by a virus. In attempt to visualize intracellular virus and to obtain further information on cell changes, electron microscope studies were made on liver or kidney biopsies of infectious hepatitis and other disease patients.

During the past one year, biopsy materials from liver or kidney in cases of infectious hepatitis, serum hepatitis, hemolytic jaundice, non-specific hepatitis, and familial non-hemolytic jaundice have been studied in thin sections with the electron microscope. The cases of infectious hepatitis and serum hepatitis have showed cytoplasmic particles suggesting a virus etiology, but it is not possible to show characteristic particles in other cases.

Braunsteiner et al. (7) have demonstrated small particles ranging from 420 to 580 A in size in cases of serum hepatitis, and larger cytoplasmic bodies 1,800 to 2,500 A in diameter in cases of probable epidemic hepatitis. Essen and Lembke (8), and Morzychi et al. (9) had previously observed virus-like particles 1,800 A in diameter in duodenal secretions, but no sections had been studied. Their work was apparently regarded as unconfirmed. Cachera and Darnis (10) showed particles about 1,800 A in size in one case of relapsing viral hepatitis. Tujimura et al. (11) noted small cytoplasmic particles in a case of hepatitis. They did not discuss these as being viral-like. Gueft (12) demonstrated innumerable fine cytoplasmic oval particles in the liver about 400 x 600 A in size in 3 cases of serum hepatitis. Bearcroft (13-14) has demonstrated that the cytoplasmic matrix of some liver in cases of infectious hepatitis contains numerous granules which measure approximately 21.5 mμ in diameter, and suggested that they may be possibly represent the causative virus. Recently, Rightsel et al. (15), and Taylor et al. (16) have reported that the infectious particle is 12 to 18 mμ in diameter on the tissue culture of serum hepatitis virus.

Several authors have reported cytoplasmic particles different in size and structure in infectious hepatitis, as mentioned above. The present author's findings are rather similar to those of Bearcroft (13-14). But, further study

will afford a further approach to the clarification of the particles found in cases of infectious or serum hepatitis.

5. Summary

Biopsy materials from human liver or kidney in cases of infectious hepatitis, serum hepatitis, hemolytic jaundice, chronic non-specific hepatitis, and familial non-hemolytic jaundice were studied in thin sections with the electron microscope. The cases of infectious hepatitis and serum hepatitis showed cytoplasmic particles suggesting a virus etiology, but it was impossible to identify characteristic particles in other cases. The ultrastructure of cellular alterations accompanying infection by the virus and other hepatotoxic agents was demonstrated and discussed in detail.

6. Literatures cited

1) Yasuzumi, G.: Electron microscopy and x-ray scanning microanalysis of needle biopsy material from human liver, J. Cell Biol., 14, 421, 1962.

2) Dames, W. Th.: MOUSE LIVER LYSOSOMES AND STORAGE, A morphological and histochemical study, Drukkerij "Luctor et Emergo"-Leiden, 1962.

3) Neefe, J. R., and Stokes, J., Jr.: An epidemic of infectious hepatitis apparently due to a water borne agent, J. Amer. Med. Assoc., 128, 1063, 1945.

4) Neefe, J. R., Stokes, J., Jr., Baty, J. B., and Reinfold, J. G.: Disinfection of water containing causative agent of infectious (epidemic) hepatitis, J. Amer. Med. Assoc., 128, 1076, 1945.

5) Findlay, G. M., and Willcox, R. R.: Transmission of infective hepatitis by faeces and urine, Lancet, 1, 212, 1945.

6) Stokes, J., Jr.: The control of viral hepatitis, Amer. J. Med., 32, 729, 1962.

7) Braunsteiner, H., Fellinger, K., Pakesch, F., und Neymayer, A.: Weitere elektronenmikroskopische Beobachtungen bei Virushepatitiden, Klin. Wschr., 36, 379, 1958.

8) Essen, K. W., and Lembke, A.: Kann der Erreger der Hepatitis epidemica als identifiziert gelten?, Klin.

Wschr., 5, 317, 1956.

9) Morzychi, J., Taylor, K., and Juskievic, E.: Attempts to discover the virus of hepatitis epidemica in allantoic fluids of chicken embryos and in tissue culture fluid by means of the electron microscope, Bull. Inst. Mar. Trop. Med. (Danzig), pp. 49 and 50 (Nov.) 1956.

10) Cachera, R., and Darnis, F.: Examen du foie humain (Prelevé par ponction-biopsie) au microscope electronique, Rev. Franc. Etudes Clin. Biol., 1, 296, 1956.

11) Tujimura, H., Azuma, S., Kitamura, H., and Takeyama, T.: Fine structure of human liver cells, Electron Microscopy (Jap.), 6, 50, 1958.

12) Gueft, B.: Viral hepatitis under the electron microscope, Arch. Path., 72, 75, 1961.

13) Bearcroft, W. G. C.: The liver in infectious hepatitis, Nature, 190, 549, 1961.

14) Bearcroft, W. G. C.: Electron-microscope studies on the liver in infectious hepatitis, J. Path. Bact., 83, 383, 1962.

15) Rightsel, W. A., Keltsch, R. A., Taylor, A. R.: Status report on tissue-culture cultivated hepatitis virus, J. Amer. Med. Assoc., 177, 671, 1961.

16) Taylor, A. R., Rightsel, W. A., Bogga, J. D., and McLean, I. W., Jr.: Tissue culture of hepatitis virus, Amer. J. Med., 32, 679, 1962.

7. Explanation of Figures

Figs. 1 to 3 show electron micrographs of thin sections of the liver in hemolytic jaundice.

Fig. 1 depicts the nucleus (N) in which the karyoplasm is fine granular in character and the nuclear envelope is not clear in its double structure. Mitochondria (M) in small size, light spots (negative images of glycogen), lipide (L), and aggregate of ferritin particles (FP) appear in the cytoplasm. Numerous ribosomes (arrows) are found surrounded the mitochondria (M). A peculiar body (PB) can be seen at the upper right corner. X 44,000.

Fig. 2. The field is almost occupied by the nucleus (N). Ribosomes (arrows) appear isolated or attached to outer surfaces of cisternal elements of the endoplasmic reticulum. X 39,000.

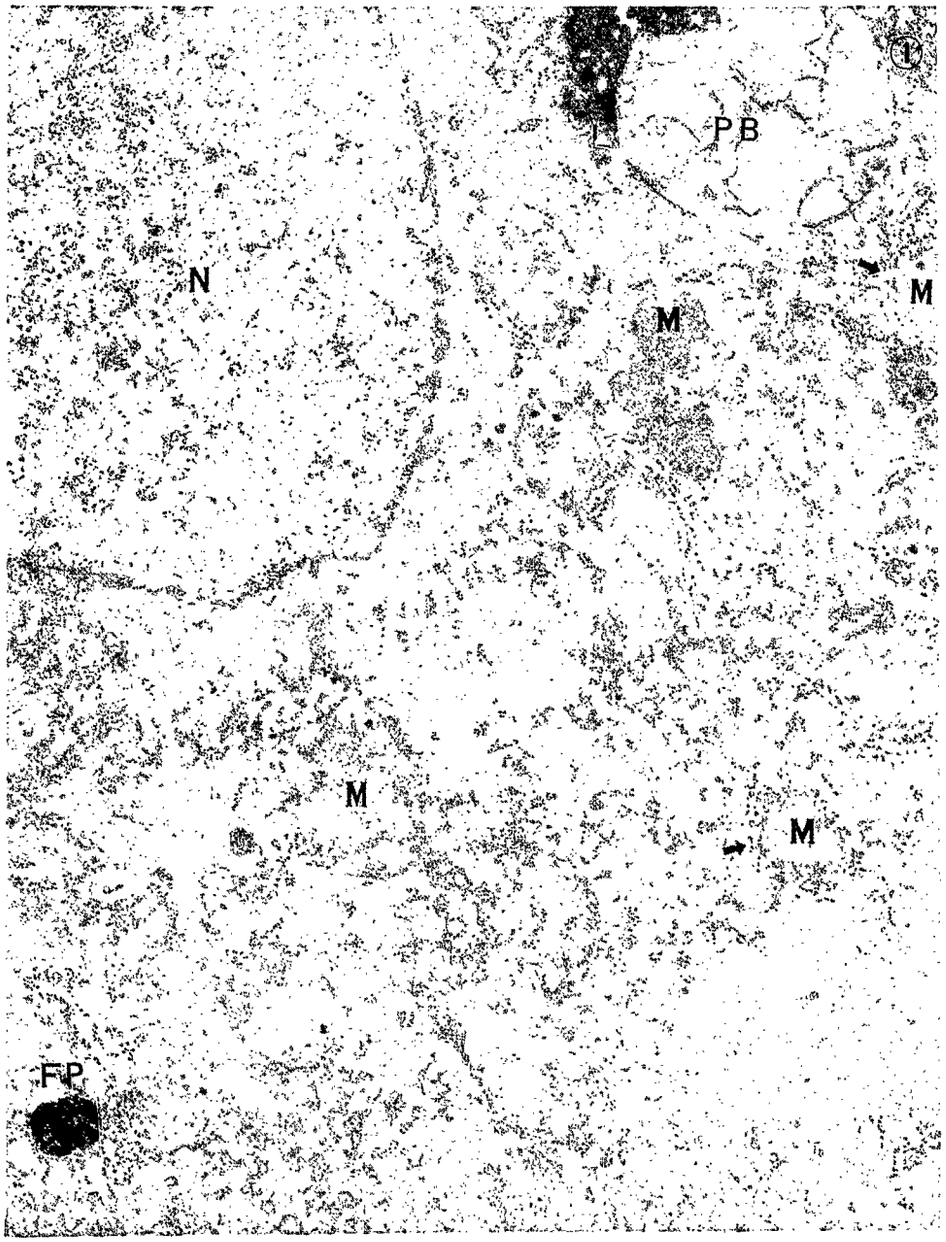
Fig. 3. Several dense bodies (DB) with or without limiting membrane, and ribosomes (arrows) appear in the cytoplasm. X 36,000.

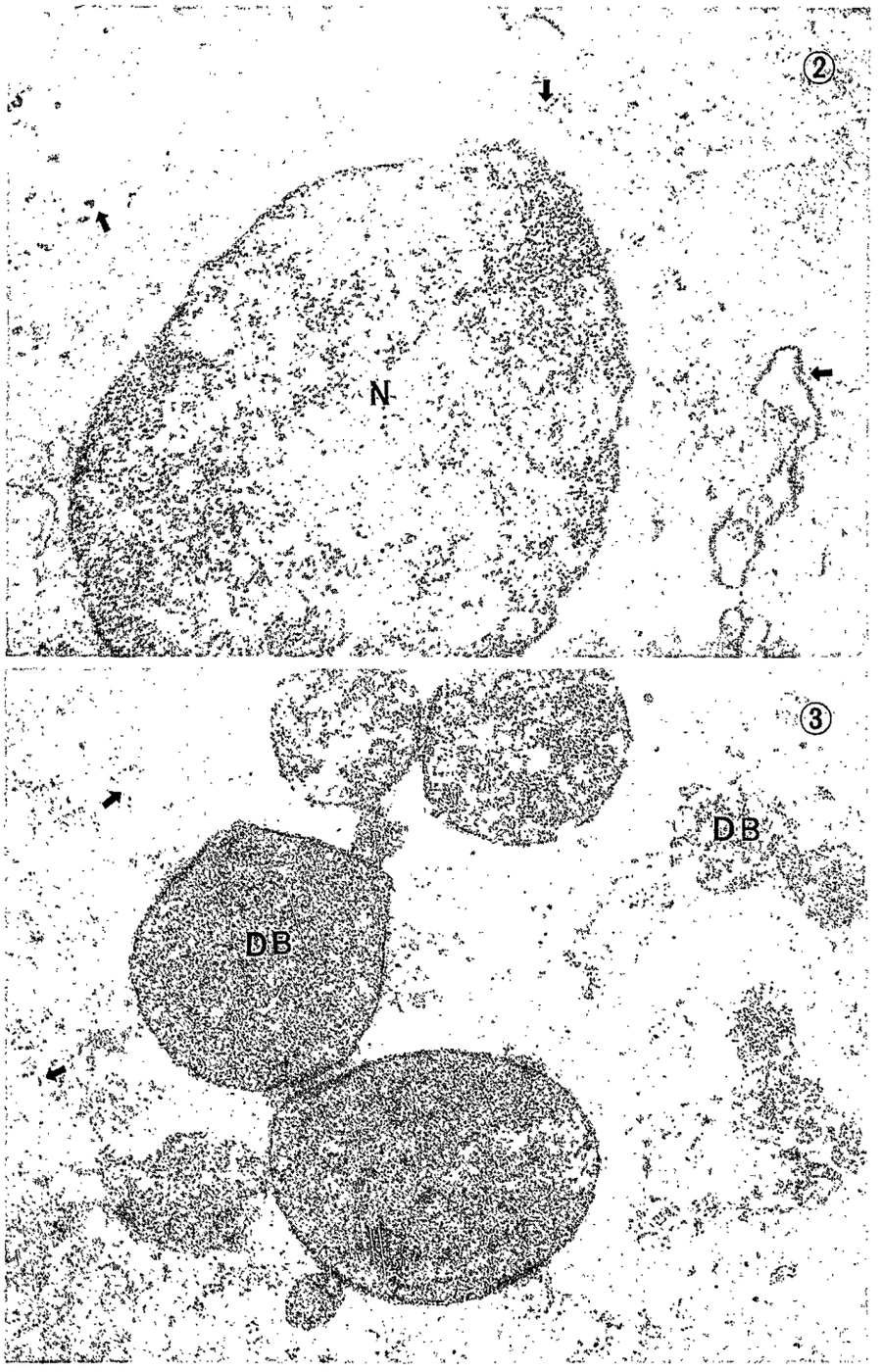
Figs. 4 to 6 demonstrate electron micrographs of thin sections of the liver in chronic non-specific hepatitis.

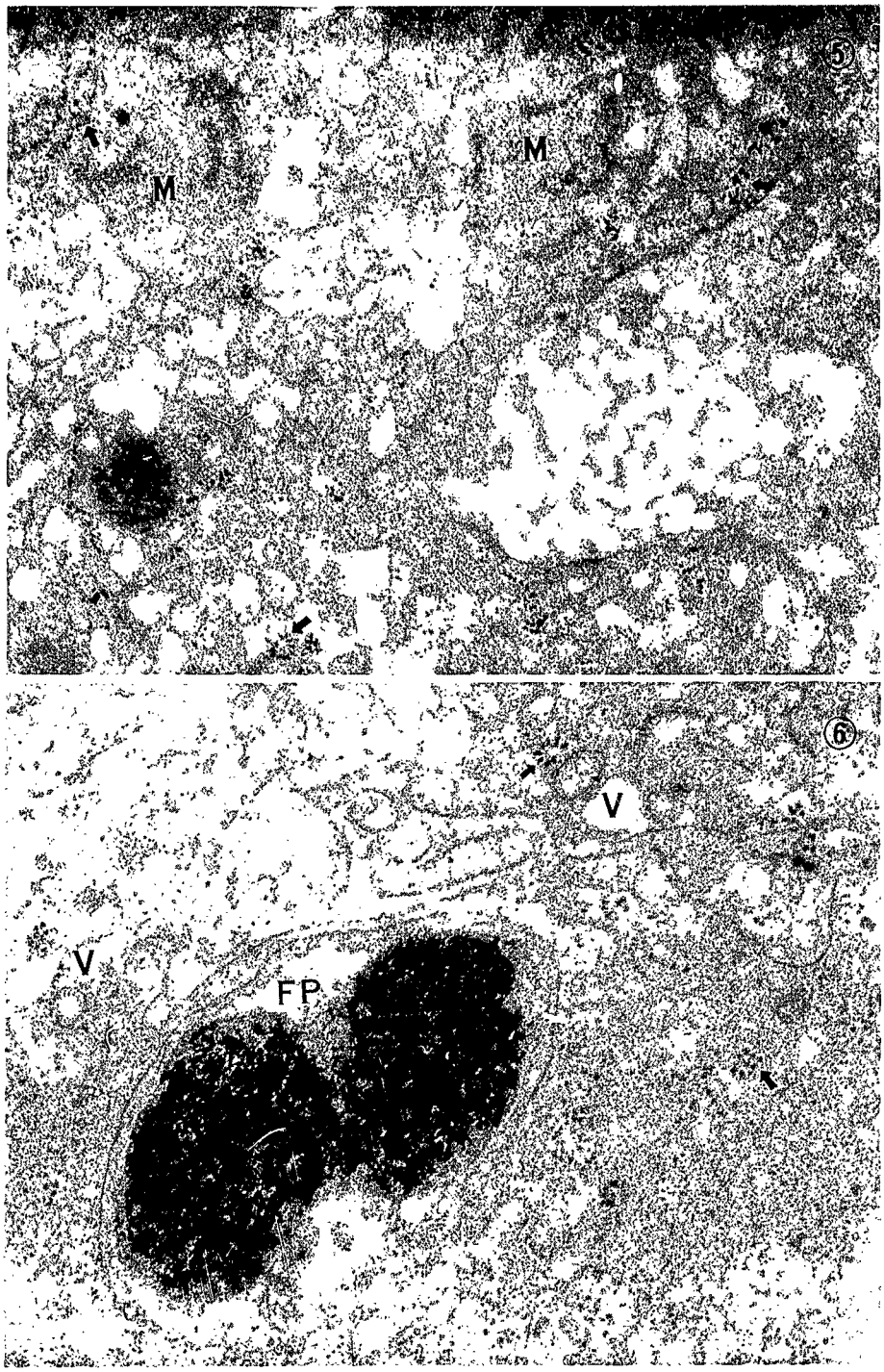
Fig. 4 shows the Kupffer's stellate cell (KC) in which the nucleus (N), nucleolus (NI), and the cytoplasm with numerous smooth and rough surfaced endoplasmic reticulum (ER) as well as with isolated ribosomes are clearly visible. X 40,000.

Fig. 5. Ribosomes (arrows), lysosome (LY), mitochondria (M) and negative images of glycogen granules are visible in the cytoplasm. X 37,000.

Fig. 6. Two aggregates of ferritin particles (FP), ribosomes (arrows) and vesicles (V) are found in the cytoplasm. X 45,000.


PB
N
M
M
M
M
FP


②
N
③
DB
DB


⑤
M
M
⑥
V
V
FP